FILARIASIS VECTOR CONTROL

A study of the control of DDT-resistant *Culex quinquefasciatus*, vector of Bancroftian Filariasis, in British Guiana

by

P.F. de Caires, M.B., Ch.B., Edin.(1938), M.P.H.

Johns Hopkins (1948).

30 APR 1952

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24 April, 1952

Dr. P. F. de Caires, Constant Spring P.O.St.Andrew, Jamaica

Dr. M. G. Candau, Assistant Director

Thesis entitled "Filariasis Vector Control".

We enjoyed reading your thesis entitled "Filariasis Vector Control" and we send you our heartiest congratulations for this fine work.

We are returning your thesis now by registered air mail because Dr. Paoliello has a copy of it.

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24-4-52

Appended is a second vol:

Giglioli, George. Malaria, filariasis and yellow fever in British Guiana. 1948.

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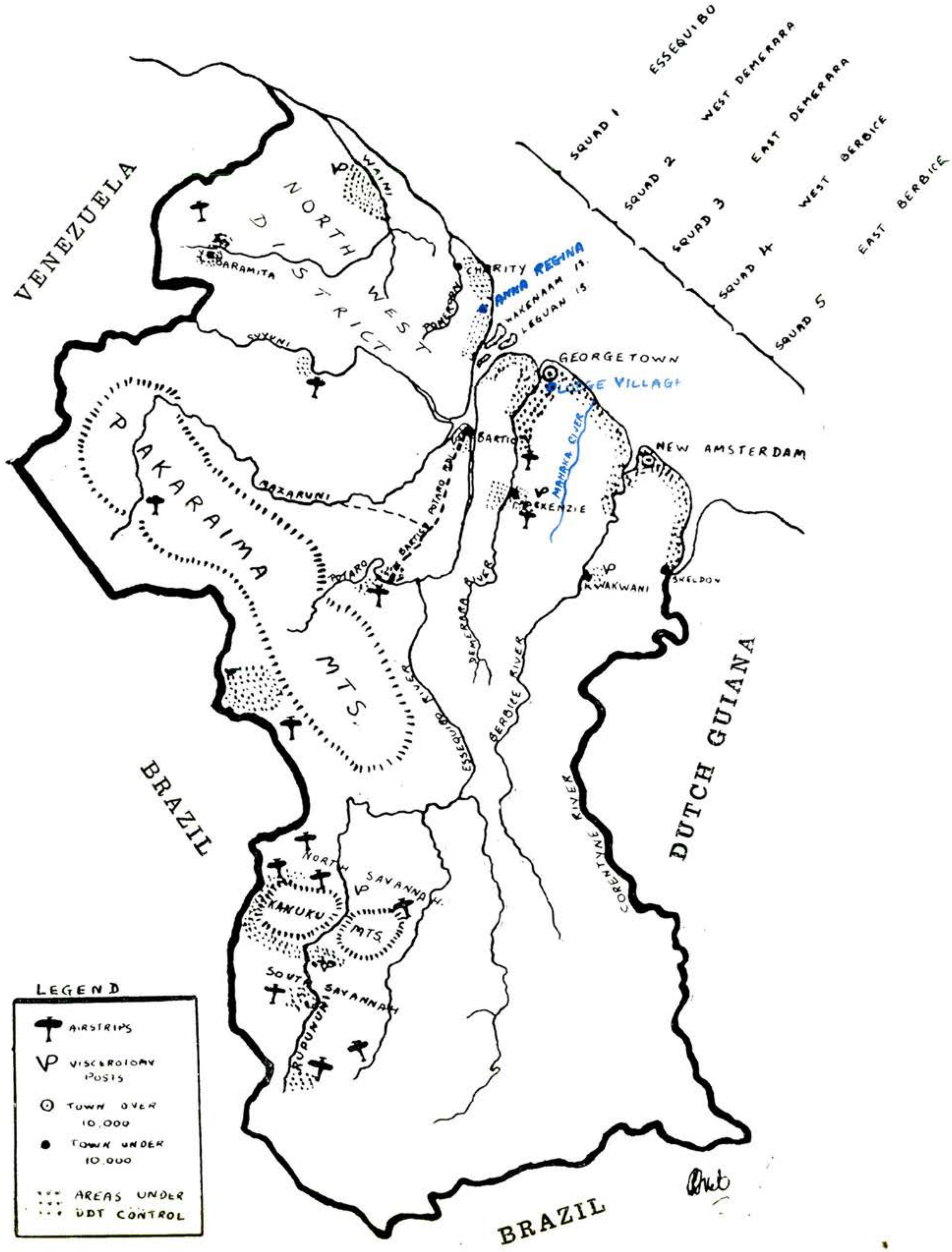

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I INTRODUCTION

British Guiana is the only British territory in South America. It lies on the north coast between 2° and 8° north latitude (see map). It has an area of roughly 90,000 square miles and a population of approximately 400,000. The thermometer seldom goes above 90°F or below 72°F. The average annual rainfall is in the vicinity of 100 inches; there are two main rainy seasons, November-January and April-June, but considerable variations occur.

Prior to 1945 the three most important public health problems were all mosquito-borne diseases, namely malaria (Anopheles darlingi), filariasis (Culex quinquefasciatus) and the threat of urban yellow fever (Aedes aegypti). Control measures were different and under separate jurisdictions. The advent of DDT in 1945[1] offered a possible solution to all three problems by the single measure of applying DDT residues to the interior of houses, all three vectors being domestic species.

The success of this method in eradicating A.darlingi[2] and Aedes aegypti[2,3,4] led to the amalgamation, in January 1947, of the Malaria Research Unit and the Yellow Fever Control Service into a single Mosquito Control Service, which also undertook the control of C.quinquefasciatus by extending residual spraying to include pit-latrines.

II CULEX RESISTANCE TO DDT

Early in 1946, in an experiment by the writer[3] to compare DDT residual house-spraying with the classical methods of Aedes aegypti control, it soon became obvious that there was a marked difference between the reactions of C.quinquefasciatus and Aedes aegypti to DDT residues. While the latter disappeared completely 11 weeks after spraying, C.quinquefasciatus was not effectively controlled. This was confirmed later on by other workers. Giglioli[5] found that DDT residual house-spraying had little effect on C.quinquefasciatus. In St. Croix, Virgin Islands, Kohler, Brown and Williams[6] began a filariasis control program in late 1946; the incidence of C.quinquefasciatus was initially reduced by 50% and, as a side effect, Aedes aegypti was eradicated. Periodic retreatments of 200 mgms. DDT per sq. ft. have been continuous in St. Croix since 1947, but although a check in 1950[7] confirmed the absence of Aedes aegypti, it was found that the C.quinquefasciatus House Index, i.e. the percentage of houses inspected found with breeding or adults, was 100%. Floch[8] in French Guiana, has also found that C.quinquefasciatus breeding persists while Aedes aegypti and A.darlingi disappear. Culex resistance to DDT was also reported in Venezuela[9] and California.[15] All of these reports are based on essentially similar techniques, whereby DDT is applied residually to the interior of houses at a dosage of approximately 150-200 mgms. per sq. ft.

DDT resistance in other insects.

The existence of DDT-resistant house flies was reported by Sacca[10] and by Lindquist and Wilson,[11] among others. This subject was pursued by Wilson and Gahan,[12] who reported that DDT-resistant house flies were also resistant to chlorinated camphene, chlordane, pyrethrum, rotenone and thanite; this finding was not confirmed by Mainwarring,[13] who got better results with benzenehexachloride against roaches and flies than with DDT. Van Grumbergen[31] found that benzenehexachloride gave better control than DDT, especially against arachnids, but the effect was not as prolonged. In our experience DDT-resistant C.quinfasciatus, house flies, fleas, roaches and ants have responded well to other insecticides, e.g. chlordane and benzenehexachloride. On the other hand, the mite, Bdellonyssus bursa, and an experimentally produced strain of C.quinquefasciatus, which were resistant to benzenehexachloride, responded to DDT. Our results show that resistance to one insecticide does not necessarily mean resistance to others. Further, the responses of _C.quinquefasciatus_ to DDT used as an adulticide and a larvicide, varied considerably.

III POSSIBLE CAUSES OF CULEX RESISTANCE TO DDT RESIDUES

1. Excitant and repellent action of DDT.

Reeves, Washburn and Hammon,[14] investigating the effectiveness of residual DDT deposits on adult _Culex_ mosquito populations, found that while a dosage of 200 mgms. per sq. ft. produced a 95% reduction of _C.tarsalis_ in houses, the population over the whole area was only moderately reduced. They also reported[15] that, as regards Western Equine Encephalitis virus infection rates in _Culex_ mosquitoes and chickens, residual DDT deposits, plus larviciding, gave an appreciable reduction in the number of resting mosquitoes in chicken houses, but no significant decrease in the incidence of infection in birds. This finding could be explained by a reaction similar to that reported by Muirhead-Thomson[16] for _A.gambiae_ and _A.melas_ in West Africa; in this experiment house-spraying with pyrethrum and DDT both caused an increase in outside, as opposed to inside, resting. The same author[17] reported the repellent action of DDT against _A.gambiae_ and the absence of this effect with benzene-hexachloride. Kennedy[18] suggested that DDT, by irritating the mosquito and causing it to leave the treated surface prematurely, failed to kill because of a sublethal exposure. The present study confirmed the excitant action of DDT against C.quinquefasciatus but produced no evidence that the drug acted as a true repellent.

2. Incorrect Dosage.

Weathersbee and Arnold[19] found 160-200 mgms. per sq. ft. to be more efficient than lesser concentrations. Quarterman[20] showed that the optimum dosage lay in the region of 200 mgms. per sq. ft. and that applications of 400, 600 and 800 mgms. per sq. ft. were not more effective. Estimations of dosages applied in British Guiana[2] gave a range of 100-300 mgms. per sq. ft. and an average dry deposit of 150-160 mgms. per sq. ft.

3. Loss of DDT after application.

Loss of DDT after application was emphasised by Fay, Buckner and Simmons,[21] who showed that there was a definite loss of residue as a result of the movement of insects over the treated surface, and also by penetration into the wall. Clapp, Fay and Simmons[22] stressed the absorbent powers of different surfaces and the resultant variations in effectiveness. They reported that DDT applied to rough wood, fabrics, well dried paints and rubbing varnish gave better results than on whitewash, spar varnish and gloss or flat paints dried for 1-7 weeks; there was almost no effect on linoleum and adobe. The explanation given was the varying ability of the different materials to hold the DDT crystals on the surface. Barlow and Hadaway[23] pointed out that there was less absorption of wettable DDT and benzenehexachloride powders than of solutions or emulsions, on mud blocks. In the present study the vast majority of the surfaces treated were rough wood, unpainted or papered board and old paints, to which technical DDT of proven quality and known content of p.p. isomer[≠] was applied in a 5% solution in kerosene. Wettable powder[≠≠] was used on adobe surfaces.

In wooden houses built on pillars there was a definite loss of DDT residue by vibration; displaced crystals could be found on the floor at the foot of the walls. The amount was not appreciable per surface area and tests on the walls themselves indicated an apparently unimpaired insecticidal action. In over-crowded houses many parts of the bedroom walls were covered by clothing hanging on nails, hooks, etc. It is difficult to assess this factor accurately but it must have impaired the efficiency of the residue to some extent at least. This effect would, however, be fairly stable in all phases of the work.

≠ Manufactured by Geigy & Co.,Ltd., Manchester (72%); E.I.Du Pont de Nemours Co.,Delaware (80%) and Dickenson & Son, Lancashire (80%).

≠≠ Manufactured by the American Cyanamid Co.

4. Intervals between applications.

Applications of the insecticide were made at intervals of 6-11 months, with an average of 7.5 months. The question of the continuous effectiveness of the application during these periods of time is beyond doubt. O'Hara[24] reported that treatment of latrine interiors leaves them insecticidal for one year. Downs, Colorado and Gahan[25] found that in the third season after application, the ratio of mosquitoes in rooms treated 29 months previously, to untreated rooms, was 1.35 : 14.8. Working with A. quadrimaculatus, Fay and Simmons[26] reported that an exposure of 180 minutes gave an 84% kill after 68 weeks. Our own experience has shown that DDT residues are insecticidal for at least 21 months after application.[27]

5. Impaired effectiveness of retreatments.

This study has shown that DDT residues, applied to previously treated surfaces, did not give as good results as initial applications. Fay and Simmons[26] reported that there was a higher rate of loss of effectiveness during the first 20 weeks than from week 20 to week 56. Complaints against repeat-sprayings became so widespread that Quarterman[20] carried out a series of tests in Savannah. He was of the opinion that reports of the failure of retreatments of DDT to give as good results as initial applications were not borne out. He did find, however, that retreatments of a higher dosage, viz. 400 mgms. per sq. ft., gave the best results. The same author reported[28] that further treatments did not increase the residual effectiveness of DDT and chlordane in anopheline control. Parkin & Green,[29] using house-flies and an exposure of ½-3 hours, found that there existed a critical value for the product of the concentration and deposit of DDT. They reported that toxicity increases with the deposit per unit area and the concentration of the solution, even though the amount of DDT applied remained constant; they felt that there was less penetration with higher concentrations. They also found that boards with a good carpet of crystals, when rubbed vigorously with a cloth, were far less toxic to flies, but after 10 days storage - although crystal formation could still not be detected - toxicity was regained. They concluded that the persistence of toxicity over 18 months was closely bound up with crystallization of the deposits and the power of regeneration.

Our results indicate a definite decrease in the effectiveness of retreatments as compared with the initial application. It was found that crystallization occurred much more quickly in subsequent sprayings with kerosene solutions, perhaps due to the "taking up" of old crystals and the creation of a super-saturated solution and excessively rapid crystallization. In this connection Davidovici, Levinson & Reuter[30] have found that a better action is obtained, against flies and mosquitoes, the longer the DDT can be kept in solution. They reported that DDT-lanoline residues were superior for that reason. We have also found that DDT reapplied to previously treated surfaces, from which the old DDT had been removed by vigorous brushing, gave far superior results than routine retreatments.

However, as will be seen, routine retreatments gave an unimpaired lethal effect on laboratory-bred _A. darlingi_ and _Aedes aegypti_, but not against strains of _C. quinquefasciatus_ from areas in which this species had had no previous contact with DDT, indicating a fundamental difference in the initial reaction of _C. quinquefasciatus_ as compared to either _A. darlingi_ or _Aedes aegypti_.

6. _Habits of the Mosquito_.

It is obvious that the more domestic the species, the more susceptible it will be to insecticidal residues applied to the interior of houses, except where the insecticide exercises a repellent action.

Aedes aegypti and _A. darlingi_ are at one end of the scale, being extremely domestic and therefore exposed to frequent contact with the residue. Further along could be placed _A. albimanus_ and _A. aquasalis_; these species are not domestic but a varying percentage of females come indoors for a blood meal and rest there. Stephens & Pratt,[32] working in Puerto Rico, reported that DDT residual house-spraying had no effect on the overall population of A.albimanus as estimated by animal bait and light-trap collections, yet malaria incidence declined as a result of the killing off of the adults which entered houses to feed and were therefore the likeliest to become infected. We had the same experience in the North West District of British Guiana with A.aquasalis,[33] where a malaria epidemic subsided dramatically while the adult and larval findings actually increased. Gillette[34] in Trinidad has been able to reduce the spleen rates from A.aquasalis-borne malaria tremendously, yet mosquito breeding persists. At the other end of the scale are such wild species as A.bellator, A.albitarsis, Aedes taeniorhynchus, and Mansonia titillans, upon which DDT residues have no measurable effect.

C.quinquefasciatus is definitely a domestic species in British Guiana and in the study area they were found breeding very close to houses. The adults frequented dwellings of all types in large numbers, day and night.

7. A true species resistance.

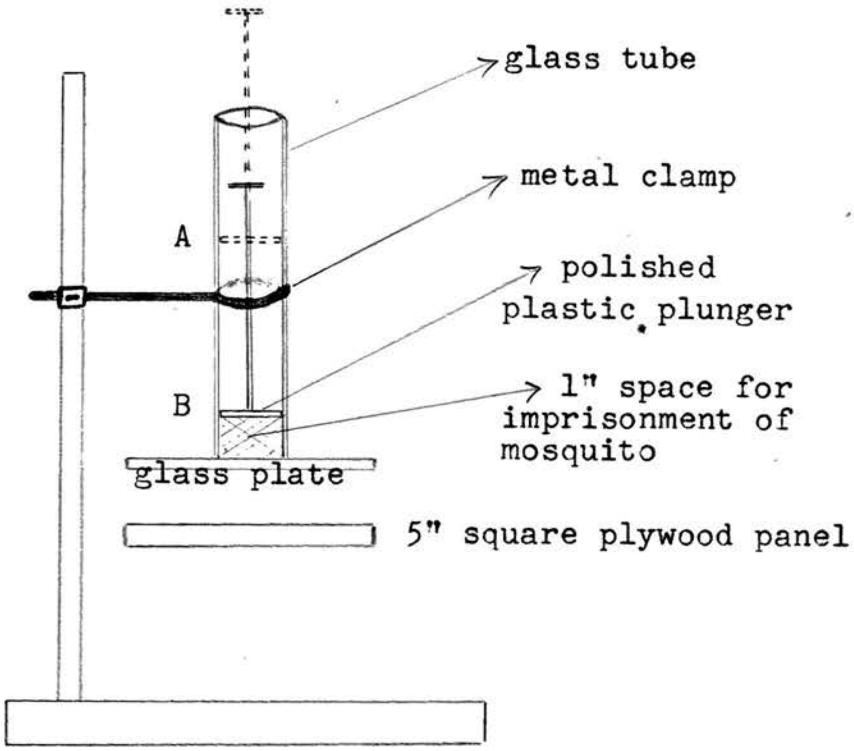
The initial experience[3] by the writer in British Guiana with previously unexposed strains of C.quinquefasciatus and Aedes aegypti certainly indicated a basic difference in the response of these two species to DDT residues. The other findings previously mentioned[5,7,8] support this hypothesis. Ribbands[35] also reported that he found DDT more effective, and for twice as long, against _A.minimus_ than against _A.vagus_ and the Culicines.

The results of the present study show that this natural resistance can be built up by constant contact with the insecticide, perhaps by the production of a super-strain through elimination of the weak. But our results also show that once this chain of constant exposure is broken, even for a single generation, by withholding contact completely in the laboratory, or applying another insecticide to the DDT treated surface in the field - thus breaking the chain - the build up was lost.

The theory of survival of the fittest would explain the decrease in overall mosquito population, without adequate control, after each retreatment. The finding in the laboratory that smaller adults were more susceptible than larger ones of the same age is not out of keeping with this view.

Figure 1.

Apparatus for exposure of mosquito



IV MATERIALS & METHODS

The general plan of action was, first of all, to determine, in the laboratory, the responses to DDT and other residues of strains of C.quinquefasciatus which had had previous contact with DDT and strains which had not, and then to apply the information gained to control measures in the field. Tests were also carried out with laboratory-bred A.darlingi, Aedes aegypti - no specimens being available in the field as a result of the successful eradication programme - and Aedes taeniorhynchus, for comparison.

1. Laboratory.

Using the apparatus shown in Fig. 1, two to three day-old freshly blooded adult females were exposed to the following residues on plywood panels:-

(a) 6-month old DDT, applied as a 5% solution of technical DDT, containing 80% p.p. isomer, in commercial kerosene at a dosage of 150 mgms. per sq. ft. (referred to as "old DDT").

(b) 1-week old DDT applied as above (referred to as "fresh DDT").

(c) fresh DDT upon old DDT.

(d) fresh DDT applied to a previously treated surface from which the old DDT had been removed by vigorous brushing with a hard brush.

(e) 8-week old benzenehexachloride (BHC), applied as the Liquid Concentrate≠ containing 20% gamma BHC diluted with 47 volumes of commercial kerosene, at a dosage of 11 mgms. gamma BHC per sq. ft. (referred to as "old BHC").

≠ LG.240, manufactured by Imperial Chemical Industries Ltd.

Figure 2.

Individual Exposure Data

Run No. 3 Date 9/8/49 Species aegypti Surface BHC in kero.

Panel No. 14 Age of Panel 8 weeks

Time				Contact				Activity				Clean			
Min.	Sec.	On	Off	F	M	H	P	Still	Walk	Fly	Probe	A	L	W	Misc. Info.
00	00	x		x	x			x							
	15		x							x					
				front legs	middle legs	hind legs	proboscis					antennae	legs	wings	

(f) 1-week old BHC, applied as above (referred to as "fresh BHC").

(g) fresh BHC applied on old DDT.

(h) 1-week old Chlordane, applied as a 2% solution in commercial kerosene, at a dosage of 50 mgms. per sq. ft. (referred to as "fresh Chlordane").

(i) 8-week old chlordane, applied as above (referred to as "old chlordane).

(j) 1-week old chlordane plus DDT, applied together in commercial kerosene at dosages of 50 and 150 mgms. per sq. ft. respectively (referred to as "fresh chlordane plus DDT").

(k) 8-week old chlordane superimposed on 18-week old DDT, in dosages as at (j) above, so that the residues were aged 8 and 26 weeks respectively at the time of exposures (referred to as "old chlordane plus DDT").

In addition, there were "control" exposures to untreated panels. Residues were applied by a dropper for precise dosage.

An adult, very lightly anaesthetised with chloroform, was placed on a clean glass plate and covered with the glass tube with the plunger at position A. When the adult had fully recovered the treated plywood panel was quickly substituted for the glass plate; the plunger was then pushed down and maintained at a distance of one inch from the treated panel, position B. The behaviour of the adult was recorded on the form shown in Fig. 2. As soon as the period of exposure was completed the panel was removed and the glass plate replaced; the adult was again lightly anaesthetised by the insertion of a piece of chloroformed gauze against the perforated plastic plunger. It was then transferred to a small wire-frame cage covered with moist cotton, containing a prune and watchglass with wet filter paper, where it was kept for 48 hours; survival or death was recorded at the end of that time. It was a routine observation that adult C.quinquefasciatus would rest on the treated panel in preference to the smooth surfaces of the glass tube and plastic plunger, even after they began to show irritability. The other species tested, particularly A.darlingi, tended to move away from the treated surface more frequently, but for very brief intervals. This observation is in keeping with reports on the fumigant and repellent action of DDT and BHC upon anopheles by such workers as Gebert,[36] Gabaldon[37] and Field.[38]

Recently blooded females were used because they were hardier than either unfed females, or males, and more uniform results were obtained with them. After this technique was mastered five adults were exposed simultaneously, transferred to a larger cage and survival or death recorded.

2. Field.

Work was then undertaken to demonstrate the effect of the different insecticides on C.quinquefasciatus under field conditions. At Lodge Village, on the south-eastern edge of the capital city of Georgetown, kerosene-soluble and water-wettable preparations of DDT and BHC, and a solution of chlordane in kerosene, were applied to pit-latrines, both as a larvicide and as a residual adulticide, and to dwelling houses, as residues. Applications were made with Cooper stirrup-pumps and B.G. nozzles, the technique being as described by Giglioli.[2] At Anna Regina, a village on the Essequebo coast, tests were carried out to compare DDT and BHC, both as initial applications and retreatments. At settlements on the Mahaica River the effects of DDT and BHC were compared on mosquitoes and other house-frequenting insects.

The writer had manufactured a DDT-soap, prepared by dissolving 45% technical DDT in warm coconut oil, stirring into a neutral soap and allowing to solidify. The resulting solid contained 12½% DDT by weight and was used as a "residual" larvicide in pit-latrines in Newtown, a village in the eastern suburbs of Georgetown and in the street drains in Georgetown itself. The dose applied was one ounce of the soap to 100 gallons of water, i.e. a DDT concentration of approximately 62 parts per million if dissolved immediately, but much lower in actual fact as the soap took a long time to dissolve, losing about 2% of its weight per day in still water.

The results in the field were assessed by standard methods, viz. "flit" adult captures at night in bedrooms≠, hand captures during daylight, larval searches in and around houses during the day and dipping with a long-handled ladle in pit-latrines.

≠ Between 7-8 p.m., after closing doors, windows and other openings the room was treated with a pyrethrum space spray and the knockdown collected on white cloth spread over the floor, etc.

V RESULTS

1. Laboratory Results.

A. Choice of Adult

The selection of adult females, given a blood meal about three hours before exposure for 25 minutes to a fresh 150 mgm. per sq. ft. DDT residue, was based on the following results:-

TABLE 1

3-day old adult *C.quinquefasciatus* from pupae collected in central Georgetown where DDT had not previously been used.

No. and type of adults	No.surviving at the stated number of hours after exposure.							
	6	12	18	24	30	36	42	48
25 males	23	11	3	1	1	0	0	0
25 unfed females	22	17	14	6	5	5	1	1
25 blooded females	25	25	21	18	14	7	7	6

Exposures were made in "runs" of 5 adults each. The chances of the differences between unfed and blooded females after 24 and 48 hours, being due to chance alone are 1 in 500 and 1 in 28. The differences are therefore statistically significant.

TABLE 2

Variation in the response of <u>C.quinquefasciatus</u> adult blooded females, from pupae collected in central Georgetown, according to age. Exposures in runs of 5 adults each.

No.	Age in days	No.surviving at the stated number of hours after exposure.							
		6	12	18	24	30	36	42	48
25	1	22	17	11	10	6	3	1	0
25	2	25	25	20	19	13	9	9	5
25	3	25	24	18	15	13	10	8	6
25	4	25	25	21	17	12	7	7	4
25	5	25	23	23	16	14	9	9	6

There being no significant difference in the results for adults aged between 2 and 5 days, 2 to 3-day olds were used.

TABLE 3

Variation in the response 2-3 day old adult unfed female <u>C.quinquefasciatus</u>, from pupae collected in central Georgetown, according to size, arbitrarily divided into "small" and "large".

No.	Size	No.surviving at the stated number of hours after exposure							
		6	12	18	24	30	36	42	48
25	small	20	16	10	6	1	2	1	0
25	large	23	18	14	13	7	8	5	5

The chances of the differences at 24 and 48 hours being due to chance alone are 1 in 50 and 1 in 29 respectively. The differences are therefore significant.

B. Choice of length of exposure

Using 2 to 3-day old C.quinquefasciatus adult blooded females, from pupae collected in Central Georgetown, assays were made to determine a convenient time of exposure to a fresh 150 mgm. per sq. ft. DDT residue. The results are shown in Table 4 and a period of 25 minutes was selected for routine use.

TABLE 4

Response to varying lengths of exposure.

No.exposed	No.of survivals 48 hours after stated period of exposure, in minutes							
	5	10	15	20	25	30	35	45
5	5	5	4	3	3	2	2	0
5	5	4	4	2	2	2	2	1
5	4	4	4	3	3	3	1	1
5	4	4	4	2	2	2	2	1
5	5	4	2	2	2	2	2	1
TOTALS: 25	23	21	18	12	12	11	9	4

C. Responses of the different species to various insecticides tested.

Panels were prepared, as previously described, with given dosages of the insecticides to be tested, and 2 to 3-day old blooded adult females of the different species were given a 25-minute exposure to each treated panel and to an untreated panel as a control.

A strain of C.quinquefasciatus was obtained from Lodge Village, the houses of which had had six applications of DDT residues, in July 1945, February 1946, September 1946, March 1947, January 1948 and January 1949. This strain will be referred to as the "Lodge strain" and will be written "C.quinquefasciatus L.S." in the tables. Another strain was obtained from central Georgetown, where DDT had never been applied; it will be referred to as the "Georgetown strain" and will be written "C.quinquefasciatus G.S." Whenever the Lodge strain was to be tested, pupae were collected in the field a few days previously and used as required, so as to permit this strain to maintain constant contact with the DDT residues. The Georgetown strain was colonised in a separate building and maintained without contact with DDT.

A.darlingi and Aedes aegypti were taken from colonies maintained by Giglioli[39] at Sophia. Aedes taeniorhynchus pupae were collected as required from salt marsh breeding grounds outside Georgetown, from an area in which DDT had never been applied.

Whenever pupae were collected from the field, the adults from them were not utilised unless over 90% of the collection survived in the laboratory. In only three instances did the mortality exceed 9%, two of which were due to contamination of the field containers≠, one with lysol and one with dilute acid; the third remained unexplained.

≠ an earthen drain and a metal drum.

Groups of 5 adults were exposed together and transferred to cotton covered cages for 48 hours, at the end of which time the deaths and survivals were recorded. The cotton covers were then removed and thoroughly washed, first in gasoline and then with soap and hot water. In the beginning these covers were checked for residual insecticidal action against male adult Aedes aegypti; in the face of uniformly negative results this practice was discontinued.

The experimental panels, when not in use, were stored in open vertical racks against an inner wall, unexposed to sunlight or direct currents of air. No attempt was made to control temperature and humidity.

As will be easily seen, the two strains of Culex quinquefasciatus reacted very differently to the other species, the latter being virtually wiped out by all residues with the exception of "old Chlordane", while a large number of Culex survived. The fresh Chlordane and fresh BHC residues gave the best results against Culex. It will also be clearly seen that DDT residues had less of an effect against the Lodge Strain, which had had previous contact with the insecticide.

TABLE 5

Reactions of 2 to 3-day old adult blooded females of different species to various insecticide residues.

Panel treated with	Dose mgms. per sq.ft.	Age of residue in weeks	Total survivals of 20 runs, each of 5 adults, 48 hours after exposure: C.quinquefasciatus L.S.	C.quinquefasciatus G.S.	Anopheles darlingi	Aedes aegypti	Aedes taeniorhynchus
old DDT	150	26	46	34	0	0	10
fresh DDT	150	1	30	22	0	0	0
fresh DDT on old	150	1/26	40	30	0	0	0
fresh DDT on surface from which old DDT brushed off	150	1/26	26	18	0	0	0
old BHC	11	8	20	18	2	6	8
fresh BHC	11	1	6	10	0	0	0
fresh BHC on old DDT	11	1/26	6	12	0	0	0
fresh Chlordane	50	1	0	0	0	0	0
old Chlordane	50	8	84	90	72	82	92
fresh Chlordane plus DDT	50/150	1	0	0	0	0	0
old Chlordane plus DDT	50/150	8/26	54	32	4	0	14
Untreated Controls	-	-	100	98	90	98	100

The individual records of each run of five adults of the different species are listed in Tables 6-10.

TABLE 6

C.quinquefasciatus L.S.

Panel	Survivals of each run of 5 adults																				Total Survivals of 20 runs
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
old DDT	4	1	2	4	2	3	1	2	4	1	3	1	2	2	1	4	1	2	3	3	46
fresh DDT	0	1	4	0	1	4	0	2	3	3	1	1	1	0	3	3	2	1	0	0	30
fresh DDT on old	2	2	1	4	1	3	2	1	0	3	4	3	1	1	3	2	3	2	2	0	40
fresh DDT on surface from which old DDT brushed off	2	3	0	0	0	2	2	0	2	0	3	1	2	1	0	2	1	2	1	2	26
old BHC	1	1	2	1	2	1	1	1	0	2	1	1	1	0	1	2	1	0	0	1	20
fresh BHC	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	6
fresh BHC on old DDT	1	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	1	0	1	6
fresh Chlordane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
old Chlordane	5	4	5	4	3	5	3	3	5	5	4	4	4	5	4	3	4	5	5	4	84
fresh Chlordane plus DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
old Chlordane plus DDT	1	4	3	3	3	2	4	3	1	3	4	3	3	1	2	4	3	1	3	3	54
Untreated Controls	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	100

The chances of the difference between the results for old DDT and fresh DDT being due to chance alone are 1 in 50. The figure for the difference between fresh DDT and fresh DDT on old, is 1 chance in 33. Comparison of the figures for fresh DDT on old, and fresh DDT on a surface from which the old DDT had been brushed off show that there is but 1 chance in 50 of the difference being due to chance alone. All the differences noted are therefore significant.

TABLE 7

C.quinquefasciatus G.S.

Panel	Survivals of each run of 5 adults																				Total Survivals of all 20 runs
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
old DDT	2	1	3	2	1	1	3	1	1	3	2	1	2	1	2	2	1	2	1	2	34
fresh DDT	1	0	2	1	0	2	2	0	2	0	0	2	1	1	1	2	2	2	0	1	22
fresh DDT on old	1	2	2	1	1	1	3	0	1	3	2	1	3	1	3	0	1	3	0	2	30
fresh DDT on surface from which old DDT brushed off	2	1	2	0	1	2	0	2	1	0	0	1	1	1	0	0	1	0	1	2	18
old BHC	0	1	1	2	0	0	2	0	2	1	1	0	1	2	0	2	2	1	0	0	18
fresh BHC	1	0	0	1	0	0	2	0	0	1	0	0	0	1	0	0	1	1	1	1	10
fresh BHC on old DDT	1	0	0	1	1	2	1	0	1	0	0	1	0	1	0	1	1	0	1	0	12
fresh Chlordane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
old Chlordane	4	5	5	4	5	5	4	5	4	4	4	5	4	5	5	5	5	4	5	3	90
fresh Chlordane plus DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
old Chlordane plus DDT	2	3	2	1	2	1	1	1	1	0	1	2	1	2	2	1	3	2	1	3	32
Untreated Controls	5	5	5	4	5	5	5	5	5	5	5	5	4	5	5	5	5	5	5	5	98

The chances that the difference between the results for old and fresh DDT being due to chance alone are 1 in 62. The comparative figure for the difference between fresh DDT on old, and fresh DDT on a surface from which the old DDT had been brushed off, is 1 chance in 62; for a comparison of fresh DDT and fresh BHC, the figure is 1 chance in 100. All differences are therefore significant.

TABLE 8

Anopheles darlingi

Panel	Survivals of each run of 5 adults																				Total Survivals
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
old DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh DDT on old	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh DDT on surface from which old DDT brushed off	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
old BHC	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2
fresh BHC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh BHC on old DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh Chlordane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
old Chlordane	3	2	3	4	4	3	4	5	3	4	4	3	4	4	4	3	4	4	3	4	72
fresh Chlordane plus DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
old Chlordane plus DDT	1	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	4
Untreated Controls	4	5	5	4	5	5	4	3	4	5	5	4	4	5	5	4	5	5	4	5	90

TABLE 9

Aedes aegypti

Panel	Survivals of each run of 5 adults																				Total Survivals
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
old DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh DDT on old	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh DDT on surface from which old DDT brushed off	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
old BHC	1	0	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	0	1	6
fresh BHC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh BHC on old DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh Chlordane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
old Chlordane	3	3	4	5	4	4	5	5	3	4	4	5	4	3	5	3	4	4	5	5	82
fresh Chlordane plus DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
old Chlordane plus DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Untreated Controls	5	5	5	5	5	5	5	5	5	4	5	5	5	5	4	5	5	5	5	5	98

TABLE 10

Aedes taeniorhynchus

Panel	Survivals of each run of 5 adults																				Total Survivals
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
old DDT	1	0	0	0	1	2	0	0	1	1	0	1	0	0	1	0	1	0	0	1	10
fresh DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh DDT on old	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh DDT on surface from which old DDT brushed off	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
old BHC	0	1	0	1	0	0	0	0	1	1	0	1	0	0	0	0	2	0	1	0	8
fresh BHC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh BHC on old DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fresh Chlordane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
old Chlordane	5	5	5	4	5	4	3	5	5	5	5	5	5	5	5	3	3	5	5	5	92
fresh Chlordane plus DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
old Chlordane plus DDT	1	0	0	1	1	1	0	0	0	2	0	1	3	0	0	2	1	0	1	0	14
Untreated Controls	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	100

D. Possible repellent effect of DDT on *C.quinquefasciatus*

To determine the possibility of a repellent effect of DDT on *C.quinquefasciatus*, tests were made with batches of 50 adults in wire-frame cages covered with white gauze. The cage was so placed that three of the vertical sides were exposed to light from the windows; the fourth, "dark" side faced a blank wall covered with a dark cloth. The cages were small enough to ensure an appreciable number of resting adults on the sides at all times. The dark side of the cage proved attractive for resting and more adults were counted there than anywhere else. There was no movement in the room during the tests.

TABLE 11

Adults counted on the six sides of a cage containing 50 adult C.quinquefasciatus G.S. The very few adults in motion were counted on the side upon which they came to rest.

Time in Minutes	No.of adults resting on sides of the cage						Total active adults
			Bright Sides			Dark	
	Floor	Top	1	2	3	Side	
00	9	11	8	4	3	15	50
03	7	8	2	5	5	23	50
06	10	6	4	6	3	21	50
09	8	10	2	2	7	21	50
12	4	13	5	1	2	25	50
15	6	22	5	2	4	11	50
18	8	11	3	1	5	22	50
21	5	13	7	3	5	16	49
24	10	7	6	2	4	20	49
27	8	12	5	4	5	16	49
30	6	7	5	6	5	20	49
Maximum	10	22	8	6	7	25	
Minimum	4	6	2	1	2	11	
Average	7.5	11.3	4.7	3.3	4.4	19.1	
Mean	8	11	5	3	5	20	

The difference between the figures for the dark side and the top (the next most frequented surface) is beyond the realm of chance alone.

Having determined that the resting adults displayed a decided preference for the dark side of the cage, three similar cages were set up, each containing 50 mosquitoes. Against the dark sides of the cages three panels were so placed that there was actual contact between the panel and the gauze. One panel was treated with old DDT, one was untreated and fresh DDT was applied to the third. Readings were made simultaneously of the number of resting adults on the dark side of each cage and the results are given in Table 12.

TABLE 12

Time in Minutes	No.of adults resting on the dark side of the cages		
	Panel with old DDT	Untreated Panel	Panel with fresh DDT
00	15	19	10
03	21	22	16
06	23	20	15
09	15	16	19
12	19	17	23
15	21	26	20
18	24	21	12
21	19	13	15
24	15	19	13
27	18	16	19
30	9	22	19
Maximum	24	26	23
Minimum	9	13	10
Average	18.1	19.2	16.5
Mean	19	19	16
No.dead after 30 minutes	1	0	0

The differences recorded are not statistically significant. It can therefore be assumed that DDT exerted no repellent action on <u>C.quinquefasciatus</u> under the conditions described.

E. <u>Excitant effect of DDT on C.quinquefasciatus</u>

Individual blooded *C.quinquefasciatus* G.S. female adults were imprisoned against an untreated and a DDT-treated panel and the responses noted. As will be seen from the results in Tables 13 and 14 the adults exhibited definite signs of irritation, yet the total times spent in contact with the treated, as compared with the untreated, surface, are not significantly different. *C.quinquefasciatus* returned to the treated panel more readily than either *A.darlingi* or *Aedes aegypti*. Table 15 shows no significant difference between the G.S. and L.S. strains of *C.quinquefasciatus*.

TABLE 13

Accumulated results of 10 runs C.quinquefascuatus G.S.

Time in Minutes	DDT-treated panel Activity				Untreated panel Activity			
	Still≠	Walk	Fly	Probe	Still≠	Walk	Fly	Probe
00	8	-	2	-	7	1	2	-
03	7	1	1	1	10	-	-	-
06	9	-	-	1	8	1	1	-
09	9	-	1	-	9	-	1	-
12	9	-	1	-	10	-	-	-
15	6	1	1	2	10	-	-	-
18	7	1	2	-	7	1	2	-
21	4	1	3	2	10	-	-	-
24	9	-	1	-	10	-	-	-
27	5(1)	1	3	-	9	-	-	1
30	6(1)	1	2	-	9	1	-	-
33	3(2)	2	2	1	8	-	2	-
36	5(2)	1	1	1	9	1	-	-
39	2(3)	1	3	1	10	-	-	-
42	1(3)	2	2	2	9	1	1	-
45	2(5)	1	2	-	10	-	-	-
48	1(5)	2	1	1	8	1	1	-
51	0(5)	1	2	2	9	1	1	-
54	1(5)	2	1	1	10	-	-	-
57	1(6)	1	2	-	9	-	1	-
60	1(6)	-	3	-	9	-	1	-

≠ The figure in brackets indicates moribund adults in addition to the number listed as "still".

TABLE 14

Times spent on the treated and untreated surfaces by <u>C.quinquefasciatus</u> G.S., before any adult became moribund.

Times in Minutes	03	06	09	12	15	18	21	24	
Treated surface	140	295	437	583	729	861	995	1119	Accumulated time, in seconds of 10 runs.
Untreated surface	152	305	450	617	753	904	1061	1192	

TABLE 15

Times spent on the treated surface by the G.S. and L.S. strains of <u>C.quinquefasciatus</u>.

Time in minutes	03	06	09	12	15	18	21	24	
G.S.Strain	140	295	437	583	729	861	995	1119	Accumulated time,in seconds of 10 runs.
L.S.Strain	149	310	469	627	775	916	1040	1158	

F. <u>Crystallization times of initial and subsequent applications of 5% technical DDT in kerosene</u>.

TABLE 16

Stage of Crystallization	Time in minutes - mean of 5 runs: Initial Application	2nd Application	3rd Application
Start	85	35	15
Advanced	130	65	45
Completed	182	90	65

As will be **readily** seen, crystallization took place far more quickly when DDT was applied to a previously treated surface.

G. Laboratory produced resistant strains of *C.quinquefasciatus*.

On the assumption that the difference in the responses of the "Lodge Strain", as compared to the "Georgetown Strain", of *C.quinquefasciatus* was due to the constant contact of the former with DDT residues, successive generations of laboratory reared colonies were exposed to 25-minute contacts with DDT and BHC residues. It was found that succeeding generations became more and more resistant, but that this characteristic was not transmitted beyond a single generation. It was also clearly demonstrated that resistance to one insecticide did not necessarily confer resistance to another, under the conditions described.

TABLE 17

The response of C.quinquefasciatus to repeated 25-minute exposures to 150 mgms/sq.ft. DDT residues

Generation No.	No.adults exposed	No.survived	% survived
1	200	32	16
2	200	45	22.5
3	200	48	24
4	200	61	30.5
5	200	68	34
6	200	64	32
7	200	85	42.5
8	200	76	38
9	200	84	42
10	200	65	32.5
11	200	81	40.5
12	200	85	42.5
13	200	80	40
14	NOT EXPOSED TO DDT		
15	200	29	14.5
16	200	37	18.5
17	200	48	24
18	200	54	27

TABLE 18

The response of C.quinquefasciatus to repeated 25-minute exposures to 11 mgm/sq. ft. BHC deposits

Generation No.	No.adults exposed	No.survived	% survived
1	150	16	10.7
2	150	15	10
3	150	20	13.3
4	150	19	12.7
5	150	17	11.3
6	150	23	15.3
7	150	19	12.7
8	150	22	14.7
9	150	26	17.3
10	150	24	16
11	150	24	16
12	150	22	14.7
13	150	23	15.3
14	150	NOT EXPOSED TO BHC	
15	150	15	10
16	150	16	10.7
17	150	17	11.3
18	150	20	13.3

TABLE 19

Response of DDT-resistant C.quinquefasciatus to BHC and BHC-resistant C.quinquefasciatus to DDT. Generations 1-9 were exposed to DDT.

Generation DDT	No.exposed to BHC	No.adults exposed	No. survived	% survived
10	Not	100	40	40
11	exposed	100	38	38
12	to	100	41	41
13	BHC	100	42	42
14		100	40	40
15	1	150	18	12
16	2	150	15	10
17	3	150	18	12
18	4	150	20	13.3
19	5	150	19	12.7
20	6	150	22	14.7
Not	7	150	23	15.3
exposed	8	150	26	17.3
to	9	150	29	19.3
DDT	10	150	30	20.0
1	11	150	23	15.3
2	12	150	24	16
3	13	150	30	20
4	14	150	42	28

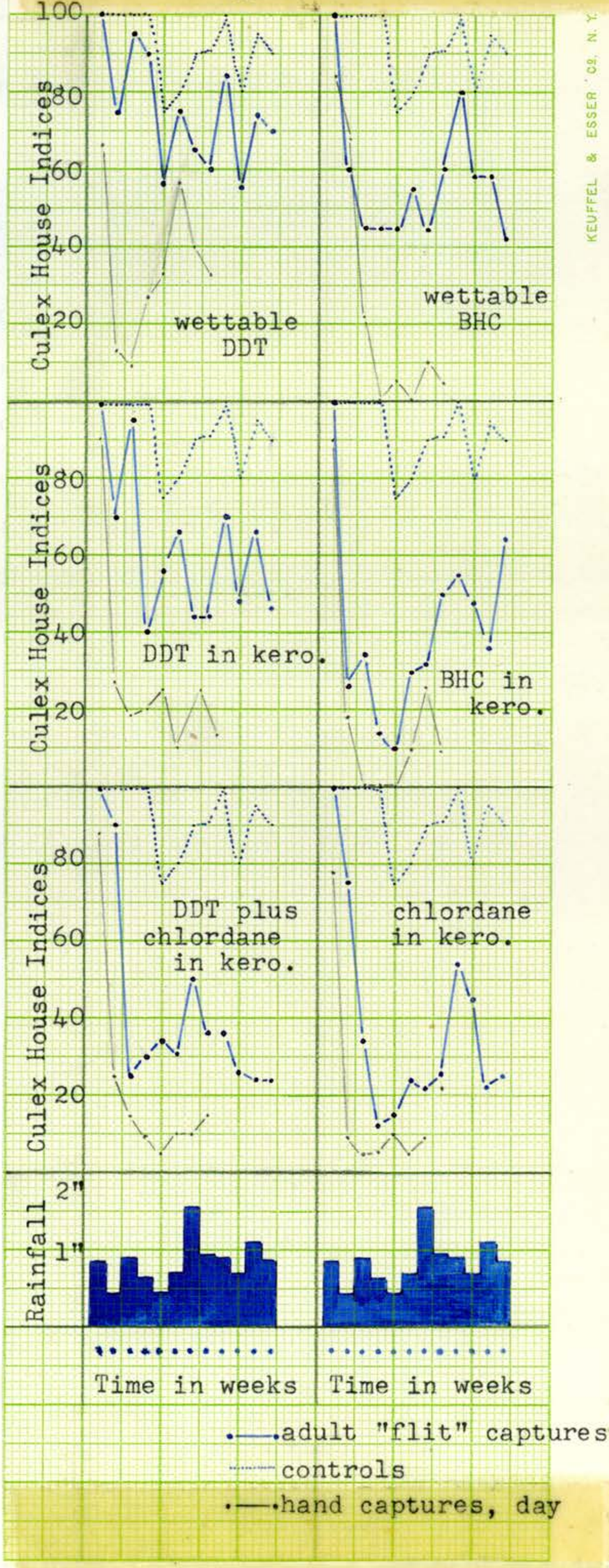
2. Field Results.

The information gained in the laboratory was now applied in the field.

A. Culex quinquefasciatus control in a previously DDT-treated area with other insecticidal residues in houses.

Lodge Village, under continuous DDT control since July 1945, was divided up into sections. After a pre-treatment survey yielded a *Culex* House Index, i.e. the percentage of houses inspected found with *Culex* breeding or adults, of 100%, various insecticidal residues were applied with the results given in Table 20:-

Figure 3.



Culex House Indices
100
80
60
40
20
wettable DDT
wettable BHC
DDT in kero.
BHC in kero.
DDT plus chlordane in kero.
chlordane in kero.
Rainfall
2"
1"
Time in weeks
Time in weeks
adult "flit" captures
controls
hand captures, day

TABLE 20

C. quinquefasciatus House Indices as determined by "flit captures" at dusk.

		Wettable DDT	Wettable BHC	5% DDT in Kerosene	2% Chlordane in Kerosene	BHC in Kerosene	5% DDT - 2% Chlordane in Kero.	Control
Dosage mgm/sq. ft.		150	11	150	60	11	150/60	-
No. houses		100	100	100	100	50	100	100
Weekly Rainfall in inches	Time in weeks.	Culex House Indices (%age houses with adult _C. quinquefasciatus_).						
0.82	0	100	100	100	100	100	100	100
0.44	1	75	61.1	70	75	27.7	89.5	100
0.86	2	95	45	95	31.6	36.8	25	100
0.62	3	89.5	44.7	40.5	12.5	10.8	30	100
0.56	4	57.5	45	57.5	15	10	32.5	75
0.71	5	75	55	66.6	27.5	30	28.9	80
1.48	6	65.7	45	42.5	25	32.5	50	90
0.92	7	62.5	50	42.5	28.2	50	52.5	90
0.90	8	84.6	80	72.5	52.5	55	57.5	100
0.70	9	51.3	56.4	47.5	45	47.5	37.5	80
1.09	10	71.8	57.5	67.5	22.5	32.5	27.5	95
0.78	11	70	42.5	42.5	25	65	25	90

These results are reproduced graphically in Figure 3.

For comparison with the results obtained by night "flit captures", "hand captures" were made, in the day time, during the first seven weeks, in the same sections of Lodge Village. The findings are given in Table 21.

TABLE 21

Culex House Indices - Hand Captures during daylight

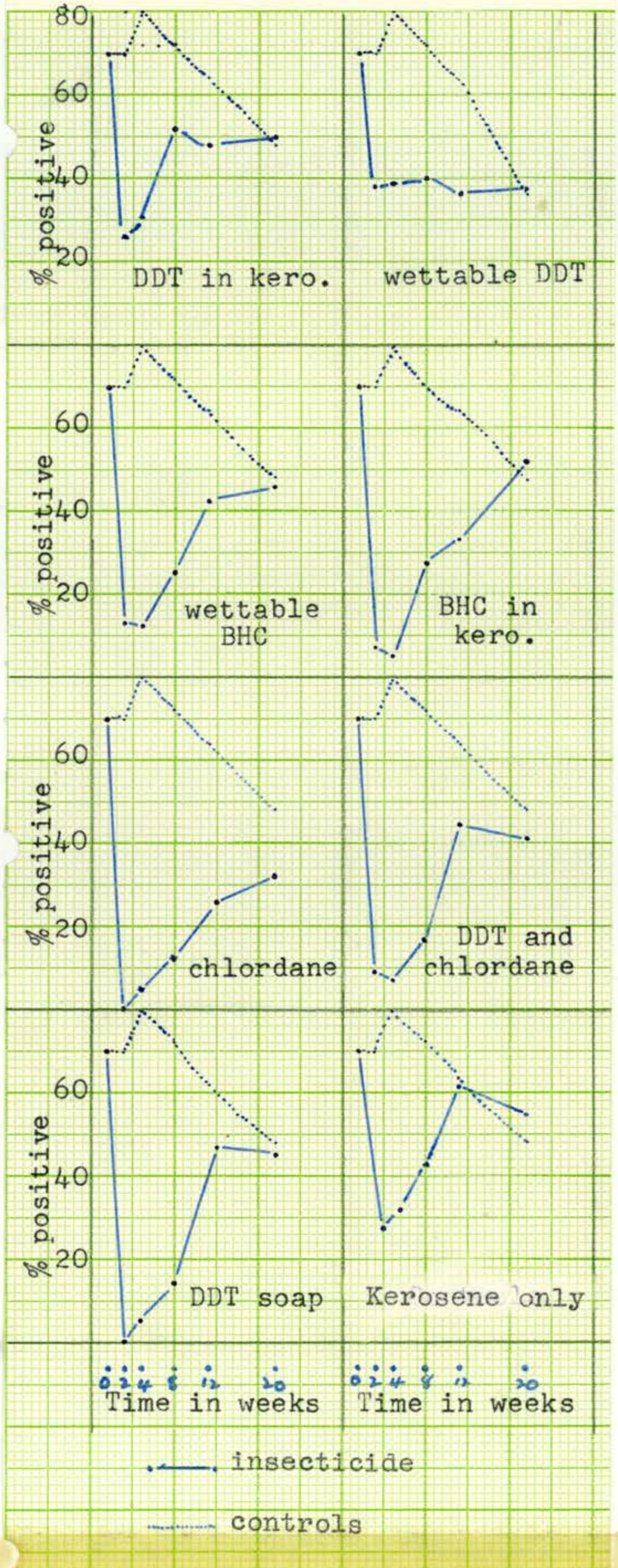
Weekly rainfall in inches	Time in weeks	Wettable DDT	Wettable BHC	5% DDT in kerosene	2% Chlordane in kerosene	BHC in kerosene	5% DDT — 2% Chlordane in kerosene	Controls
0.82	0	66.6	84.0	90.0	78.0	95.0	88.0	93.3
0.44	1	13.6	68.1	27.2	9.1	18.2	25.0	96.6
0.86	2	9.1	22.2	18.2	4.5	0	15.0	93.3
0.62	3	27.3	0	20.0	5.0	0	9.5	90.0
0.56	4	33.3	4.8	25.0	10.0	0	4.8	66.6
0.71	5	57.1	0	10.0	5.0	10.0	10.0	60.0
1.48	6	40.0	10.0	25.0	9.5	27.2	10.0	61.5
0.92	7	33.3	4.6	13.6	22.7	9.1	14.3	58.6

These results are reproduced graphically in Figure 3.

B. C.quinquefasciatus control in previously DDT-treated pit latrines with various residual insecticides at dosages previously stated.

In the sections of Lodge Village described above, pit latrines were treated with various insecticides, as a residual spray to the interior of the superstructure and the under surface of the seat.

Figure 4.



80
60
40
20
% positive
DDT in kero.
wettable DDT
wettable BHC
BHC in kero.
chlordane
DDT and chlordane
DDT soap
Kerosene only
0 2 4 8 12 20
Time in weeks
Time in weeks
insecticide
controls

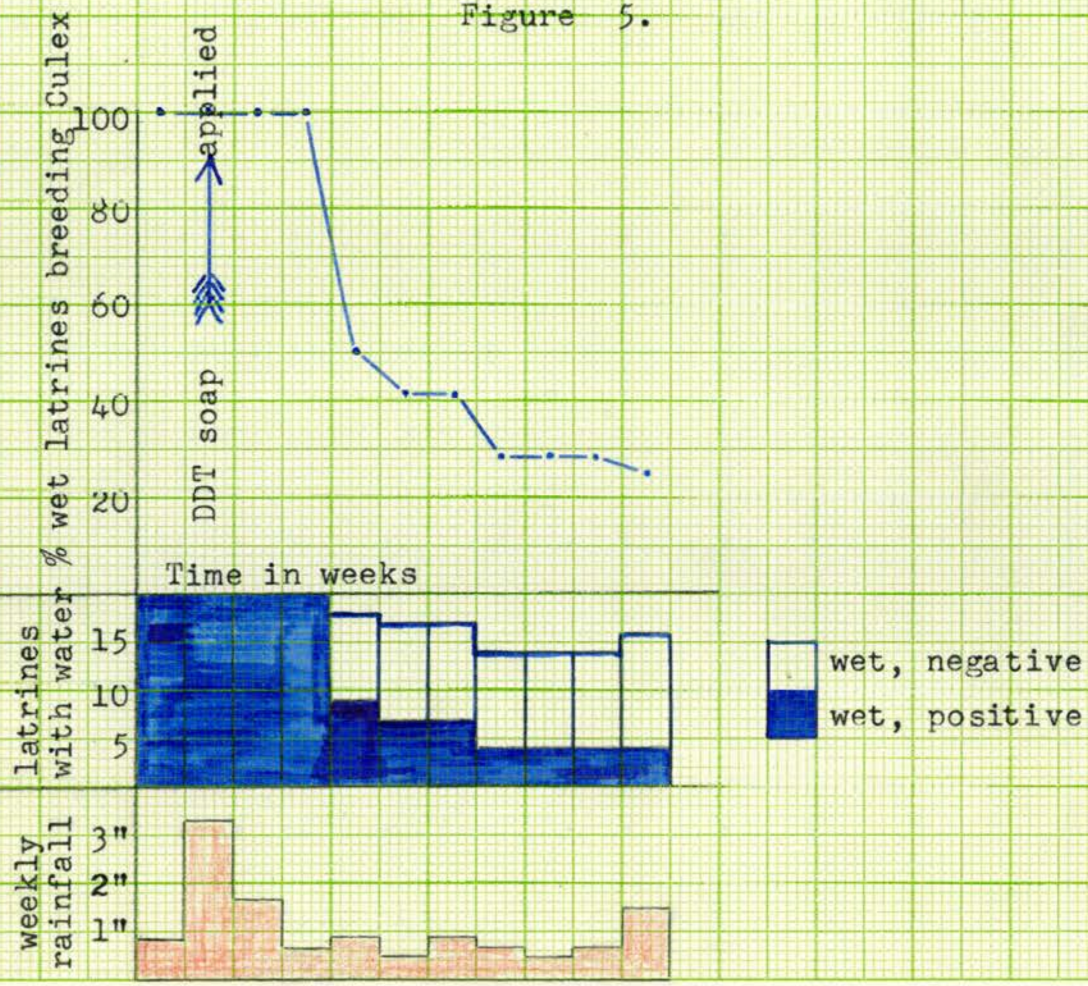
TABLE 22

Culex Breeding in Pit Latrines in Lodge Village.

Time after applications:-		2 weeks			4 weeks			8 weeks			12 weeks			20 weeks		
Insecticide	No. treated	With Water	—ve	%—ve	With Water	—ve	%—ve	With Water	—ve	%—ve	With Water	—ve	%—ve	With Water	—ve	%—ve
50% DDT in kerosene	100	19	5	26.3	22	7	31.7	36	19	52.7	27	13	48.1	34	17	50.0
Wettable DDT	100	37	14	37.8	36	14	38.8	42	17	40.5	25	9	36.0	48	18	37.5
Wettable BHC	102	29	4	13.7	33	4	12.1	40	10	25.0	29	12	41.4	60	28	46.7
BHC in kerosene	38	13	1	7.7	17	1	5.9	22	6	27.3	15	5	33.3	19	10	52.5
2% Chlordane in kerosene	98	17	0	0	24	1	4.2	33	4	12.1	30	8	26.7	50	16	32.0
5% DDT — 2% Chlordane in kerosene	101	21	2	9.5	29	2	6.9	37	6	16.2	29	13	44.8	29	12	41.4
DDT Soap	45	5	0	0	18	1	5.5	21	3	14.3	15	7	46.7	11	5	45.4
Kerosene	50	18	5	27.7	19	6	31.6	21	11	52.4	13	8	61.5	22	12	54.5
Controls	100	26	18	69.2	29	22	75.8	46	33	71.7	25	16	64.0	19	9	47.4

These results are reproduced graphically in Figure 4.

Figure 5.


% wet latrines breeding Culex
100
80
60
40
20
DDT soap applied
Time in weeks
latrines with water
15
10
5
wet, negative
wet, positive
weekly rainfall
3"
2"
1"

C. C.quinquefasciatus control in 20 pit latrines in Newtown, a suburb of Georgetown, by "DDT soap" - 12½% DDT by weight and a dosage of ½ oz. per latrine, as a larvicide. The standard volume of each pit was 45 cu. ft. Newtown had been under residual DDT control since 1947.

TABLE 23

Culex control by "DDT-soap" as a larvicide.

Time in weeks	Weekly rainfall in inches	Pit Latrines		
		With water	Breeding	% Breeding
0	0.82	20	20	100
1	3.28	20	20	100
2	1.63	20	20	100
3	0.62	20	20	100
4	0.83	18	9	50.0
5	0.44	17	7	41.2
6	0.88	17	7	41.2
7	0.64	14	4	28.6
8	0.45	14	4	28.6
9	0.66	14	4	28.6
10	1.46	16	4	25.0

These results are reproduced graphically in Figure 5.

D. Control of DDT-resistant C.quinquefasciatus by BHC, applied as a residual spray to the interior of houses at a dosage of 11 mgms/sq.ft.

At Anna Regina, a small village on the Essequebo coast, the spraying squads were greeted with open hostility when they attempted to carry out the 4th application of DDT. The householders complained that "the DDT wasn't the same as used at first". Sixty-seven houses were treated with wettable BHC (11 mgms/sq.ft.). The House Indices were obtained by daytime adult hand-captures and larval searches and are given to the nearest whole number. The density of the infestation was also assessed.

TABLE 24
C. quinquefasciatus control with BHC, in 67 houses at Anna Regina.

Previous week's rainfall in inches	Time in weeks	House Index		Adult Culex quinquefasciatus			
		Adult	Larval	Seen	Captured	Total	Average No. per house
1.82	Pretreatment Survey	100	56	1,806	527	2,333	34.8
3.04	0	BHC applied at a dosage of 11 mgms/sq. ft.					
1.91	2	42	12	70	11	81	1.2
3.33	4	25	15	35	8	43	0.6
0.64	6	50	16	100	59	159	2.4
0.44	8	44	12	166	25	191	2.8
0.62	10	59	16	136	32	168	2.5
0.71	12	33	12	67	6	73	1.1

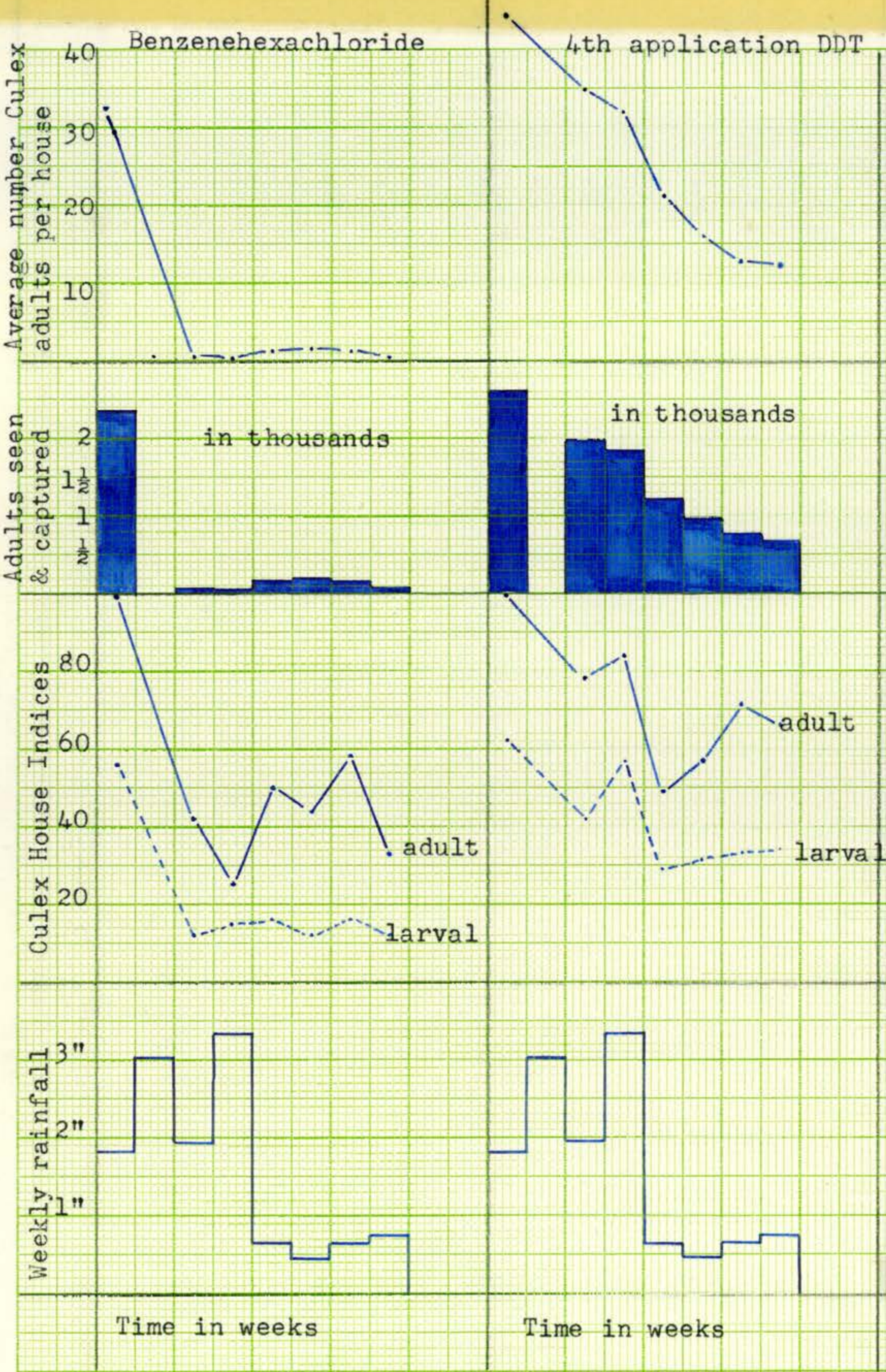

Benzenehexachloride
4th application DDT
Average number Culex adults per house
40
30
20
10
Adults seen & captured
2
1½
1
½
in thousands
in thousands
Culex House Indices
80
60
40
20
adult
larval
adult
larval
Weekly rainfall
3"
2"
1"
Time in weeks
Time in weeks

Figure 6.

In 56 houses in the same village, the 4th application of wettable DDT (150 mgms/sq. ft.) was made, with the following results:-

TABLE 25

C.quinquefasciatus control with DDT (4th application) in 56 houses at Anna Regina.

Previous week's rainfall in inches	Time in weeks	House Index		Adult Culex quinquefasciatus			
		Adult	Larval	Seen	Captured	Total	Average No. per house
1.82	Pretreatment Survey	100	62	1,823	792	2,615	46.7
3.04	0	DDT applied at a dosage of 150 mgms/sq. ft.					
1.91	2	78	42	1,664	309	1,973	35.2
3.33	4	84	57	1,605	198	1,803	32.2
0.64	6	49	29	979	224	1,203	21.5
0.44	8	57	31	773	153	926	16.4
0.62	10	71	33	604	117	721	12.9
0.71	12	66	34	576	109	685	12.2

The results in Tables 24 and 25 are reproduced graphically in Figure 6.

E. <u>The response of common household insects to DDT and BHC</u>.

Using settlements along the Mahaica River, DDT (200 mgms/sq.ft.) and BHC (11 mgms/sq. ft.) were applied as primary residues.

TABLE 26

Response to Primary Applications of DDT & BHC

Houses with	BHC (to 204 houses) Percentage houses positive		DDT (to 105 houses) Percentage houses positive	
	Before	5 weeks after	Before	5 weeks after
Mosquitoes	98.5	42.6	99.0	38.1
Fleas	23.0	2.5	30.5	1.9
Flies	98.5	6.4	97.1	20.9
Roaches	98.9	46.9	99.0	10.5
Ants	94.1	31.8	94.3	3.8
Bed Bugs	49.5	0	60.9	0
Termites	88.2	14.2	87.6	25.7
Lice	16.7	0	20.9	0
D.gallinae	46.6	2.9	59.0	0

F. The effect of reapplications of DDT on *C.quinquefasciatus*.

As has been already shown, subsequent applications of DDT residues to surfaces already treated gave less satisfactory results than initial applications of the same dosage. This could have been caused either by a resistance to the drug on the part of the insect or a reduced efficiency of the drug itself. That *C.quinquefasciatus* does develop an increasing resistance to DDT has already been demonstrated, but the loss of effectiveness was not restricted to this mosquito alone. An endeavour was therefore made to determine whether or not the fault lay, at least in part, with the drug itself.

At Anna Regina 100 previously treated houses, the occupants of which objected vigorously to their homes being resprayed on the grounds that the spraying was of no value, were divided into equal lots. Twenty-five houses had a routine reapplication of DDT in kerosene; 25 were untreated, as controls; 25 were sprayed with kerosene only; and the walls of the remaining 25 were cleaned by brisk scrubbing with a hard brush and then sprayed with DDT in kerosene. The dosage of DDT applied to the houses and pit-latrines was 160 mgms. per sq. ft.

The results were assessed by larval searches and adult captures during the day and the indices determined. The density of infestation was also evaluated. The results are given in Table 27.

Figure 7.

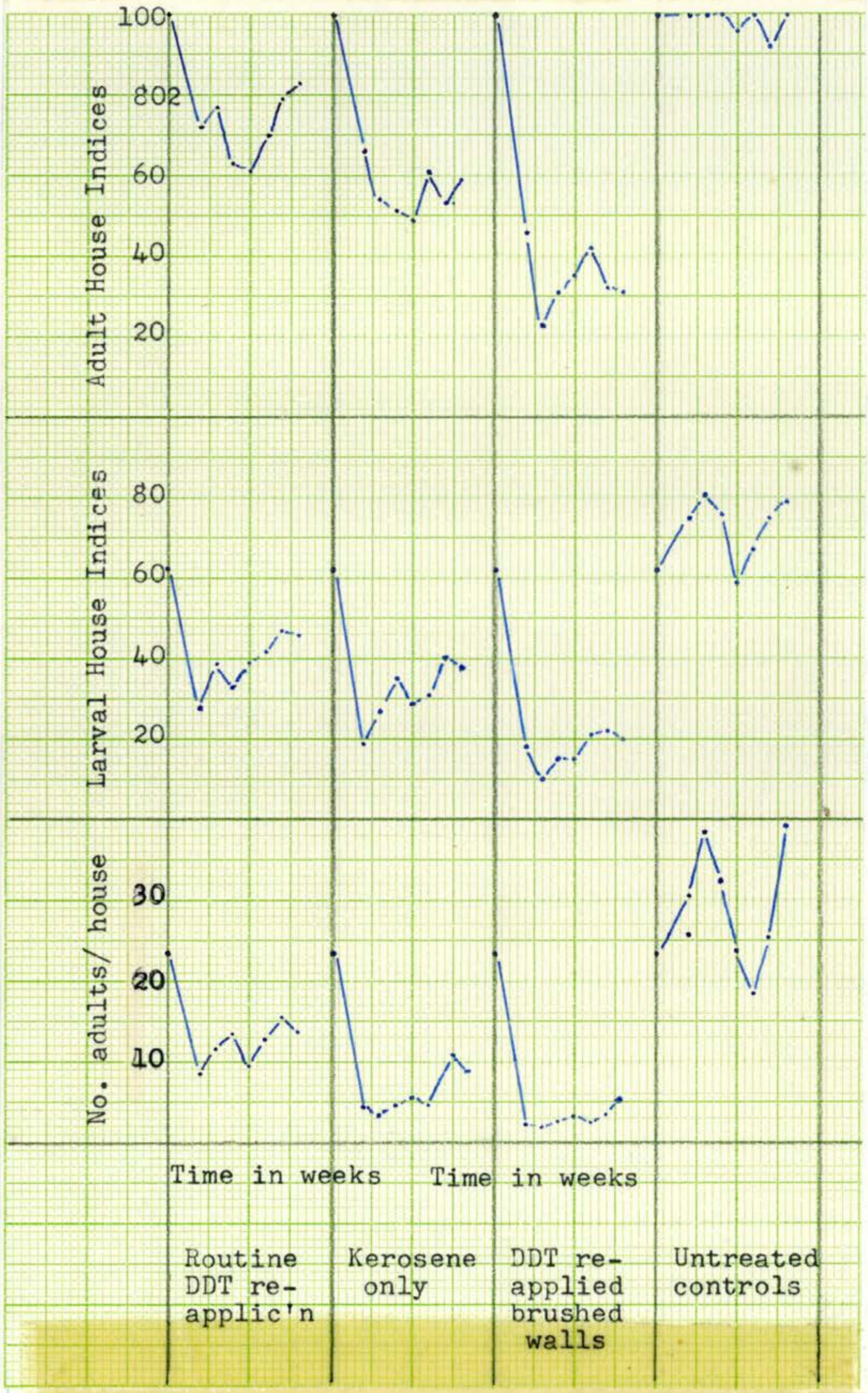

Adult House Indices
100
80
60
40
20
Larval House Indices
80
60
40
20
No. adults/ house
30
20
10
Time in weeks
Time in weeks
Routine DDT re-applic'n
Kerosene only
DDT re-applied brushed walls
Untreated controls

TABLE 27

DATE 1949	Previous week's rainfall in inches	Routine reapplication			Kerosene only			Reapplications to brushed walls			Untreated Controls		
		Adult Index	Larval Index	No.adults per house	Adult Index	Larval Index	No.adults per house	Adult Index	Larval Index	No.adults per house	Adult Index	Larval Index	No.adults per house
July 11	1.82	100	62	26.3	100	62	26.3	100	62	26.3	100	62	26.3
July 18-20	3.04	Sprayed			Sprayed			Sprayed			Controls		
Aug. 4	1.91	72	28	8.5	66	19	4.2	46	18	2.1	100	75	31.8
Aug. 18	3.33	77	39	11.8	54	27	3.5	23	10	2.0	100	81	38.8
Sept. 1	0.64	63	33	13.2	51	35	4.7	31	15	2.8	100	76	32.5
Sept.15	0.44	61	39	9.1	49	29	5.3	35	15	3.1	96	49	23.7
Sept.29	0.62	70	42	12.8	61	31	4.9	42	21	2.7	100	57	18.3
Oct. 13	0.71	79	47	15.3	53	40	11.0	32	22	3.3	92	65	25.5
Oct. 27	0.82	83	46	13.9	59	38	8.9	31	20	5.1	100	69	39.4

These results are reproduced graphically in Figure 7.

G. Resting habits of C.quinquefasciatus in DDT-sprayed and unsprayed houses.

To determine whether or not DDT residues cause any change in the resting habits of this species, adult searches were made in a small, isolated, compact rural area in which every second house had been treated with wettable DDT. The results are given in Table 28:-

TABLE 28

DATE 1949	8 unsprayed houses			8 treated houses		
	Adults seen	% indoors	% outdoors	Adults seen	% indoors	% outdoors
Aug. 8	209	84	16	239	90	10
" 15	224	81	19	217	87	13
" 22		Controls			Spraying	
" 29	193	91	9	102	78	12
Sep. 5	184	82	18	93	92	8
" 12	173	85	15	111	81	9
" 19	190	84	16	85	88	12
" 26	188	87	13	109	90	10

The differences are not statistically significant.

VI DISCUSSION

It is evident that the basic response of Culex quinquefasciatus to DDT is so different to that of Aedes aegypti and Anopheles darlingi that species eradication cannot be obtained by residual house-spraying alone, with the insecticides used. This natural resistance of C.quinquefasciatus may have been strengthened by the gradual falling off of the effectiveness of routine reapplications of DDT residues. The fact of this resistance has been noted by many observers, but the mechanism by which it is produced has not been determined. The evidence obtained in these experiments does not indicate any repellent effect of DDT and while adults exposed to DDT residues did show some degree of irritation, C.quinquefasciatus displayed no tendency to stay away from the treated surface for long. Careful searches failed to reveal any tendency to outside-resting, as opposed to the natural domestic habits of the species in entering houses and remaining there, after DDT applications.

Such possible sources of error as incorrect dosage, overlong intervals between applications and lossof residue after application were also eliminated as relevant factors.

The obvious feature of this natural resistance is that it can be increased by constant contact with the insecticide, but just as quickly lost when continuous contact is broken, even for a single generation. It is also clear that, under the conditions which obtained in British Guiana, a resistance to one insecticide did not confer resistance to another. Efforts to produce a strain with increased resistance to two insecticides, at the same time, also failed.

These characteristics offer two methods of control of DDT-resistant C.quinquefasciatus, viz. breaking contact with the insecticide before re-exposure, or applying another residue over the old one. The first method is not practical in the field where vast numbers of houses are being treated. The second yielded good results with at least two other drugs, benzenehexachloride and chlordane, and satisfactory control upon subsequent exposure to DDT.

The results obtained demonstrate the uselessness and danger of adding a second insecticide to the first, as a combined spray, when the first fails to give results. The second insecticide alone will effect control at a much lower cost; in addition, persistent use of the first drug ensures continuous contact and maintenance of a high resistance to it.

It has also been shown that DDT-resistant strains of C.quinquefasciatus displayed less resistance to DDT as a larvicide than as an adulticide. This is not surprising as contact is obviously more intimate and continuous between larva and larvicide, than between adult and insecticidal residue.

Compared to C.quinquefasciatus, Aedes aegypti and A.darlingi have shown such marked susceptibility that resistance does not develop. The greater domesticity of these two species probably plays a part in this, yet there is no denying the fundamental difference in their initial response to DDT.

VII SUMMARY & CONCLUSIONS

Culex quinquefasciatus has been shown to have a natural resistance to DDT residues on initial exposure and an increase of this resistance upon continuous exposure. Species eradication cannot be obtained by residual house-spraying alone, using either DDT, BHC or Chlordane. In sharp contrast Aedes aegypti and Anopheles darlingi are so susceptible that species eradication is attained by DDT residual house-spraying alone.

The cause of this natural resistance is not known, but a contributing factor may be the loss of effectiveness of succeeding applications of the drug superimposed on old residues. DDT did not exercise any repellent action on C.quinquefasciatus and its irritant effect was too mild to break contact with the residue. The dosage of, and intervals between, the DDT applications were correct.

Control of DDT-resistant strains of C.quinquefasciatus can be obtained with benzenehexachloride and chlordane. A laboratory-produced BHC-resistant strain similarly responded to DDT residues.

Control of this species, over long periods of time, should not be attempted by combined sprays, but by changing the insecticide as soon as re-applications of one residue fail to effect adequate control.

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